- 1 -

Fluorometers

The present invention relates to fluorometers, being
apparatus for producing and measuring fluorescence, whether
5   using intensity or time resolved measurements.

Epifluorescence microscopes conventionally have a linear
optical arrangement in which a sample location, beam splitter
and detector are arranged spaced, for instance vertically,
along a common axis in a first direction, with an excitation
10   light source off to one side. This arrangement dictates a
minimum height constraint so as to provide room for
fluorescence light emitted from a sample location to be
collimated by a lens system, passed through the beam
splitter, filtered to remove wavelengths other than that of
15   the fluorescence and finally to be focussed onto the
detector. Such a conventional arrangement is illustrated in
Figure 1 as discussed in greater detail below. In
fluorescence applications the intensity of the emitted
fluorescence is usually weak. Furthermore, the fluorescence
20   is emitted isotropically or according to a Lambertian
radiation pattern if the fluorophore is situated in a light
scattering medium such as skin. In both cases the detected
fluorescence increases with the numerical aperture of the
optical system. In the common epifluorescence set-up a large
25   numerical aperture is normally obtained by the use of a
number of lenses, which introduces reflection loss and
light scattering even for coated lenses.

It would be desirable to develop an alternative general
30   optical arrangement with reduced complexity that can be used
to reduce the necessary height of the apparatus measured from
a sample location.

- 2 -

Accordingly, the present invention now provides in a first aspect apparatus for the production and detection of fluorescence at or below a surface, said apparatus comprising:

5   a light source for directing fluorescence excitation light along a light path extending over a said surface;

a reflector having a three dimensionally curved, shell-like light reflecting interface positioned to receive
10   light from the light source passing over said surface along a portion of said light path and to reflect said light transversely with respect to said portion of the light path so as to focus said light on an illumination zone at or below said surface for stimulation of
15   fluorescence at said zone, and to collect fluorescence light emitted at said zone and to reflect and at least partially collimate said light to pass back along said portion of the light path; and

20   a detector for receiving said light emitted as fluorescence after reflection at said interface.

The 'surface' referred to above may be a physical surface defined by an interface between two different
25   materials or may be a virtual surface definable with respect to the apparatus.

The apparatus may further comprise a beam splitter reflecting light emitted by said light source to pass to said
30   reflector and receiving fluorescence light from said reflector and passing said fluorescence light to said detector.

- 3 -

Said reflector, light source and detector are preferably arranged in a generally coplanar manner and said beam splitter preferably has a planar reflective interface that lies in a plane orthogonal to the co-planarity of the
5   reflector, light source and detector.

The apparatus may further comprise an excitation filter selecting an excitation wavelength from the light emitted by the light source to pass to said reflector.

The apparatus may further comprise an emission filter
10   selecting an emitted fluorescence wavelength to pass to said detector.

The apparatus may comprise a lens focussing fluorescence light on said detector. Alternatively, a further focussing reflective interface may be used.

15   The or each said reflector interface may substantially have the form of a partial paraboloid, aspheric, toroidal, or biconic surface. Such a reflector interface may be paraboloid, aspheric, toroidal, or biconic surface. Spheric surfaces and aspheric surfaces such as hyperbolas, parabolas,
20   ellipsoids and oblate ellipsoid reflector interfaces may be defined by an equation

$$Z = \frac{cr^2}{1+\sqrt{1-(1+k)c^2r^2}}$$

wherein:

c is from 0.07 to 0.5 and k is from -1.5 to -0.7, where z is
25   the "sag" of z-coordinate along the rotational axis, c is the curvature(the reciprocal of the radius R), k is the conical constant and r is the radial coordinate. Other surfaces may be described by similar equations involving an added Taylor expansion. Similarly, equations are available for toroids,

- 4 -

e.g. faceted toriods, or piecewise linear toroids and for
biconic surfaces.

The reflector interface of the reflector directing light
to the illumination zone may include that part of a
5   paraboloid, aspheric, toroidal, or biconic surface that is
generated by the cutting of a paraboloid, aspheric, toroidal,
or biconic surface by a right circular cylinder erected
centred on the illumination zone.

Said reflector interface may preferably substantially
10   have the form of a part of a half paraboloid.

The apparatus may further include a housing containing
the light source, reflector and detector and having a base
surface containing a window for passing excitation light out
of the housing and receiving fluorescence light into the
15   housing and being for engagement in use against the said
surface at or below which said fluorescence occurs.

Preferably, said light path makes an angle of no more
than 10 degrees with a plane defined by said base surface.

The invention will be further described and illustrated
20   with reference to the accompanying drawings in which:

Figure 1 is a schematic side elevation view of a
conventional epifluorescence measuring apparatus;

Figure 2 is a perspective view of a first embodiment of
apparatus according to the present invention;

25   Figure 3 is an illustration showing the zone of a
parabolic mirror in which most energy is collected when light
is emitted from a surface over which the mirror lies;

Figure 4 is a plan view from above of a second
embodiment according to the invention;

30   Figure 5 is a perspective view of the apparatus of
Figure 4;

- 5 -

Figure 6 is a cross section on the line VI-VI' marked in Figure 4;

Figure 7 shows a modification of the apparatus of Figure 4;

5   Figure 8 shows a third embodiment according to the invention in plan from above;

Figure 9 is a graph of the variation in shape of a parabolic mirror with the conical constant; and

Figure 10 is a graph showing the variation in the ratio
10   of detected optical power and illumination power with changing conical constant.

A common set up for epifluorescence measurement is shown in Figure 1. It is designed for producing and detecting fluorescence at a sample location 10, which might be for
15   instance at or below the surface of a body or article, e.g. on a microscope slide. Typically such a surface will be horizontal and for convenience, this is assumed in the following description of the apparatus. An excitation light source 12, such as an LED, is positioned off to one side of
20   the sample location 10 and at a height h above the sample location. The LED emits excitation light 16 which is passed through an excitation filter 14 to select a desired excitation wavelength. The excitation light falls on an   ᴵ angled beam splitter 18 such as a parallel sided dichroic
25   beam splitter 18. A portion of the excitation light is reflected down at right angles towards the sample location and passes through a focussing lens system 20 comprising one or more simple or compound lenses.

Fluorescence and reflected excitation light emanating
30   from the sample location are captured by the lens system 20 and collimated into a parallel beam which passes through the beam splitter 18 to an emission filter 22, which ideally

- 6 -

removes all but the fluorescence wavelength, and from there
to a second lens system 24 that focuses the fluorescence onto
a detector 26. Like the lens system 20, the lens system 24
may comprise one or more simple or compound lenses.

5   The whole device has a total height above the sample of
h', which is around 3 x h.

It has been proposed to monitor concentrations of
chemical species in the human or animal body by fluorescence
based techniques. These would involve directing the

10   excitation light onto the skin and detecting fluorescence
emission from the skin. Relevant teachings include
WO00/02048 and WO02/30275.

We have noted that it would be desirable in such
circumstances and others to reduce the dimension h' of the

15   fluorescence detection apparatus used.

Figures 2 shows an embodiment of apparatus according to
the invention. Here, the apparatus is depicted placed on the
surface of the skin or a similar surface on which
fluorescence is to be detected. For convenience in the

20   following description, the plane of the skin will be treated
as being horizontal. A light source 32 emits light along a
path parallel to the skin surface, and determines a height h
for the apparatus which approximately equals to the total
height h' of the device.

25   The light passes through an excitation filter 34 to a
dichroic beam splitter 38 disposed in a vertical plane and is
diverted towards a half paraboloid shaped mirror reflector 40
which replaces the lens system 20 of the conventional
apparatus. The reflector focuses the light down onto an

30   illumination zone 30 on the skin surface or towards an
illumination zone just below the skin surface. The reflector
collects and collimates fluorescence emissions from the

illumination zone and directs the emitted light back to the
beam splitter, which it passes through to reach an emission
filter 42. From there, the light is focused by a lens system
44 of the same type as lens system 24 and is detected at a
5   detector 46.

It can be seen that the vertical height of the apparatus
has been reduced from approximately 3 x h in Figure 1 to just
h in Figure 2.

Generally, interference filters are used in fluorometers
10   as the excitation and emission filters. These require that
the incident light be orthogonal to the plane of the filter
if the expected wavelength passing properties are to be
obtained, because with obliquely incident light, the filter
pass band will be shifted towards shorter wavelengths.
15   Collimation of the light beams incident on these filters is
therefore required.

The paraboloid reflector 40 may be constructed in a
number of ways to produce a reflecting interface of the
desired shape. For instance, the interface may be between
20   air inside the paraboloid and a reflective concave interior
surface of a body. Alternatively, the interface may be
formed at the convex exterior surface of a solid hemi-
paraboloid member, for instance a silvered exterior of a
glass or plastics hemi-paraboloid block.

25   The whole of the illustrated apparatus will be bounded
by a housing or casing (as seen in Figure 6) having a bottom
face containing an aperture which may be filled with a
transparent window (suitably of glass, silica or in some
applications plastics) for protecting the optics against dust
30   and humidity and for allowing the passage of excitation and
fluorescence light to and from a sample in the illumination

- 8 -

zone. Said apparatus may thus be a simple opening in the
casing or an optical window.

Signals from the detector may be fed to suitable
electronic circuitry for analysis in a known manner. The LED
5 may be powered by suitable electronic circuitry as known and
the LED supply circuitry and the detected signal processing
circuitry will generally form part of an integral circuitry
for producing intensity based or time resolved (frequencey
domain) measurements of the fluorescence, e.g. in a FRET
10 (fluorescence resonance energy transfer) based assay.

The illustrated apparatus provides a high numerical
aperture for the capture of fluorescence from the
illumination zone whilst enabling a compact optical geometry
suited for use where space is at a premium, e.g. in a device
15 to be worn on the body.

Not all of the illustrated paraboloid surface is needed
in order to obtain good results. The paraboloid need not, as
shown, be arranged with its axis coincident with the surface
on which the device is to be used but may be angled up to
20 gain some additional height above the surface for the
detector optics. The beam of fluorescent light may not be
collimated entirely by the reflector but may in part be
collimated by an auxiliary lens system. The physical
illumination zone of the apparatus need not lie at the focus
25 of the reflector. The reflector interface need not be in the
form of a true paraboloid. These concepts are further
illustrated in subsequent Figures.

As shown in Figure 3, the area of the paraboloid surface
M that will receive and collimate out to the detector most of
30 the fluorescence emitted from the illumination zone will be
that defined by the intersection of a right circular cylinder
C centred on the illumination zone with the paraboloid

surface itself. The remainder of the paraboloid surface need
not therefore be present. Clearly, the smaller the cylinder,
the less light will be captured, so preferably at least as
much of the paraboloid is present as is defined by the

5   intersection with a cylinder of a radius r not less than 50%,
preferably not less than 75%, more preferably not less than
90% of the distance marked f from the origin to the focal
point of the paraboloid. The radius r of the cylinder need
not be smaller than the focal length of the parabolic mirror,

10 as in the case shown in Figure 3, but can be larger.

    Whilst in Figure 2 a lens system is shown focusing the
light onto the detector, it will be appreciated that this
could also be a reflector system instead, which might
suitably resemble that used to focus the excitation light on

15 the illumination zone. Such a system is shown in Figures 4
to 6.

    In the illustrated apparatus, the illustrated components
are as in Figure 2 except that the lens system 44 is replaced
by a part parabolic mirror 45 which resembles mirror 40 but

20 is arranged to focus the fluorescence light to the side where
the detector 46 is now positioned. As seen in Figure 6, the
apparatus comprises a housing 50 having a base plate 52 lying
on the skin and containing a window 54 as previously
described which defines the illumination zone 30. The

25 housing comprises upper and lower half shells having internal
formations to cradle and support the optical components, the
shells being secured together by screws as shown.

    As shown in Figure 7, the base surface of the housing
may lie at an angle α, preferably not exceeding 10°, more

30 preferably not exceeding 5°, to the axis of the paraboloid
surface. This will give some additional space for the
optical and electronic components but will also result in

- 10 -

some additional height at the detector end of the apparatus.
This modification may be employed both in relation to the
embodiment using mirrors at each end as shown and in relation
to the embodiment of Figure 2. Furthermore, the focal point

5   of the mirror 40 may differ from the location of the sample,
as shown on Figure 7 by the distance δ, in order to
compensate for the radiation pattern and scattering
properties of the sample or surrounding media as in the case
of detecting fluorescence under the skin.

10   As shown on Figure 8, an auxiliary lens system
comprising one or more simple or compound lenses 41
positioned in front of mirror 40 may be used to produce
further collimation.

        Similarly, the shape of the reflector may deviate from

15   that of a paraboloid in order to accommodate to the radiation
pattern and scattering properties of the sample or
surrounding media as in the case of detecting fluorescence
under the skin. If this leads to an incomplete collimation
of the fluorescence emissions, further collimation may be

20   carried out using an auxiliary lens system 41 as illustrated.

        Alternative curved surfaces that may be used include
toroidal, aspheric and biconic surfaces.

        For an aspheric surface defined by

$$Z = \frac{cr^2}{1+\sqrt{1-(1+k)c^2r^2}}$$

25   c is preferably within the range of 0.07 to 0.5 and k is
preferably within the range of -1.5 to -0.7, where z is the
"sag" of z-coordinate along the rotational axis, c is the
curvature (the reciprocal of the radius R) and k is the conical
constant and r is the radial coordinate. The shape of the

- 11 -

mirror is shown in Figure 9 for various values of the conical
constant.

In Figure 10 the influence of the conical constant on
the detected optical power is shown.

5   The illumination zone need not be a point or a circular
area but by the effect of the use of non-paraboloid reflector
shapes may be elongated either transversely or longitudinally
of the axis of the reflector.

In this specification, unless expressly otherwise

10   indicated, the word 'or' is used in the sense of an operator
that returns a true value when either or both of the stated
conditions is met, as opposed to the operator 'exclusive or'
which requires that only one of the conditions is met. The
word 'comprising' is used in the sense of 'including' rather

15   than in to mean 'consisting of'.